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Research Article

Evaluation of the Tolerance to Temperature and Salinity on Seed Germination of Three *Lamiaceae* from Algerian Scrubland

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Abstract

Global warming and soil salinity are major constraints threat speared of Mediterranean endogenous flora, however little is known about the effect of these phenomena on seed germination patterns, particularly in Algeria. Germination test under laboratory controlled conditions had been carried out using seeds of *Marrubium vulgare*, *Sideritis incana* and *Stachys ocymastrum*. Seeds were submitted at temperatures of 5, 10, 15, 20, 25, 30 and 35 °C, after that and within optimum temperatures, various sodium chloride (NaCl) concentrations of 0, 34, 68, 102 and 136 mM were set to evaluated salinity effect. Germination was more satisfactory on temperatures ranged between 15 and 25 °C. Whereas, increased or decreased temperatures from the optimum bring to germination fall. High seed germination capacity had been showed on non-saline solution. While, germination had depressed by enhanced NaCl solution up to 136 Mm in which Final Germination Percentage are either significantly decreased (for *S. ocymastrum* seeds 18% germination) or absolutely inhibited (for *M. vulgare* and *S. incana* seeds), as well Initial Germination Day are delayed. Overall, data showed that germination patterns response varies among species, given that seed germination is remarkably limited by extremes temperatures and salinity.

Key words: Germination, NaCl, Salt stress, Seeds, Temperature.

Introduction

Temperature is the most prominent environmental factor for determination periodicity and plasticity of species and plays a major role for regulating seed germination and seedling establishment [1]. Understanding the effect of temperature on seed germination is usually allowed by determining cardinal temperatures, under which optimum and limits temperatures tolerance (low and high) are assessed and geographical distributions (determination the colonization trends of plants) are predicted [2]. Temperature may affect a

number of patterns controlling seed germinability, as such membrane permeability and cytosolic enzymes synthesis [3].

Additionally, assessment tolerance range of salinity during germination is critical to better understand fate of seeds in saline soil in arid and semi-arid regions [4]. Increased salinity leads to a reduction and/or delay in germination of both halophyte and glycophyte seeds. High salt concentrations on soil thwart seed germination submitting to both osmotic and toxic effects due by either the upward movement of soil ions or consequential evaporation at the soil surface [5]. Sodium Chloride (NaCl) is commonly used to study the effect of salinity (osmotic potential) on germination as it is easy to handle. Also, NaCl is considered as one of the dominant salts in soils of arid and semi-arid rangelands [6].

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Marrubium vulgare, *Sideritis incana* and *Stachys ocymastrum* (Lamiaceae) are Mediterranean semi-arid grasses which are characterized by therapeutic effects and mainly used in traditional medicines [7, 8]. In fact, distribution of grasses in semi-arid regions is very unpredictable, with rate of colonization concomitantly linked to the temperature fluctuations, rainfall means and soil salinity [9, 10]. Moreover, Master germination patterns could approve basic knowledge about ecological requirements and can be imperatively exploited for project of conservation, restoration and soil management.

Thus, and as attempt to fill the gaps about seed germination behaviors precisely on Algerian scrubland flora, experiments were conducted to assist effect of cardinal temperatures and salinity on germination patterns of *M. vulgare*, *S. incana* and *S. ocymastrum*.

Material and Methods

Plant material

Mature seeds were collected from local populations growing on calcareous soil in Tessala Mounts (North West Algeria). This region is semi-arid with a typical Mediterranean climate, characterized by uneven rainfall events and a harsh dry summer period. Annual precipitation is between 290 to 420 mm and average monthly temperatures are between 9.4 °C and 26.6 °C.

Germination experiments

Decorticated seeds were left at ambient laboratory conditions for three months, to avoid possible after-ripening effects. Above all, seeds were surface sterilized by 1% sodium hypochlorite solution for 5 min and rinsed with distilled water. After drying, seeds were placed in fitting Petri dishes of 9-cm containing two disks of Whatman No. 1 filter papers moistened with 5 ml of distillate water or test solution. Three replicates of twenty seeds per treatment were used with which the germination tests were made in darkness. Seeds with appeared radicles were scored and removed. Seed germination was assessed daily over a 20-d period.

Effect of temperature and salinity

The effect of temperature on germination was determined in incubators set at constant temperatures of 5, 10, 15, 20, 25, 30 and 35 °C. Distilled water equal to the mean water loss from dishes was added every two days to maintain humidity. For salt effect experiments, germination was conducted at different concentrations of NaCl (0, 50, 75, 100, 125 Mm), however salinity tests were applied within each plant optimum temperature.

Germination patterns and statistical analysis

Initial Germination Day (IGD), Final Germination Day (FGD), Final Germination Percentage (FGP) and Mean Time of Germination (MTG) were patterns to determine.

MTG was calculated as follows:

$$MTG = \sum \frac{(n_i \times d_i)}{N}$$

Where n_i is the number of seeds germinated at day i , d_i is the incubation period in days, and N is the total number of germinated seeds [11].

Germination variables percentages were transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS for windows, version 20. A one-way analysis of variance (ANOVA) was performed on all results. Duncan's multiple range tests was used to estimate least significant range between means.

Results and Discussions

Temperature effects

Regarding table 1 germination was statistically significantly affected by temperature ($p<0.001$). Germination can occurred satisfactory within range of 15 to 25 °C, thereby optimum germination capacity was reached at 15 for *S. incana* (65 %) seeds and at 20 °C for *M. vulgare* (88%) and *S. ocymastrum* (62%) seeds. Conversely, germination tend to be significantly reduced by rises or decreases in temperatures from optimum range, however temperatures tolerance (low vs. high) differ among species (10 vs. 30 °C-*M. vulgare*; 5 vs. 30°C-*S. incana* and 10 vs. 35 °C-*S. ocymastrum*). Our results adhere with those of Estrelles et al. [12] who revealed that the optimal germination temperatures of semi-arid species are between 15 and 25 °C. In added, *Ballota hirsuta* (Lamiaceae) seeds had revealed same germination behavior when they have been submitted on such conditions [13]. Moreover, germination at moderate temperatures is presumed as common trait of Mediterranean plants, particularly in Lamiaceae family [14].

At 5 °C, germination of *M. vulgare* and *S. ocymastrum* exhibited complete inhibition. Even, though seeds are able to water sorption at this temperature, germination was likely prevented by either chilling injury to the embryos, metabolic inactivation or by induced secondary seed dormancy [15]. Whereas, seeds of *M. vulgare* and *S. incana* whose remained at 35 °C showed failure on germination. According to Bannayan et al. [16], temperature of 35 °C often leads to decline germination of several medicinal plants.

Overall, other germination patterns as such IGD, FGD and MTG are significantly affected by temperature regimes and response negatively on thermal stress (table 1). By increasing temperature, Initial Germination Day (IGD), Final Germination Day (FGD) and Mean Time of

Germination (MTG) fallen. As survival strategies, probably seeds can negotiate low temperatures by membrane fluidity, while the response to high temperatures implicates the participation of heat shock proteins [17].

Table 1.

Germination characteristic variables of *M. vulgare*, *S. incana* and *S. ocymastrum* seeds in response to constant temperatures (Mean \pm SE). IGD: Initial Germination Day / FGP: Final Germination Day / FGP: Final Germination Percentage / MTG: Mean Time of Germination

Temperature (°C)	Parameters			
	IGD (days)	FGD (days)	FGP (%)	MTG (days)
<i>M. vulgare</i>				
5	-	-	-	-
10	9.4 \pm 1.8 ^b	16.8 \pm 1.2 ^c	43 \pm 6 ^b	13 \pm 0.5 ^c
15	4 \pm 1.5 ^a	13.6 \pm 0.8 ^{abc}	80 \pm 4 ^a	6.73 \pm 0.52 ^a
20	3 \pm 0.6 ^a	11.2 \pm 0.8 ^{ab}	88 \pm 6 ^a	6.52 \pm 0.43 ^a
25	4.5 \pm 0.5 ^a	10.8 \pm 0.6 ^a	57 \pm 5 ^d	6.78 \pm 0.85 ^a
30	4.8 \pm 1.4 ^a	14 \pm 2.4 ^{bc}	35 \pm 4 ^b	9.06 \pm 0.91 ^b
35	-	-	-	-
F- value	31.43***	5.95*	39.64***	32.84***
<i>S. incana</i>				
5	10.6 \pm 1.5 ^a	14.6 \pm 1.2 ^a	13 \pm 3 ^a	12.7 \pm 1.2 ^b
10	7.4 \pm 0.6 ^b	15 \pm 1 ^a	45 \pm 5 ^b	11.2 \pm 1.2 ^{ab}
15	6.6 \pm 0.6 ^b	14.6 \pm 0.6 ^a	65 \pm 5 ^c	10.8 \pm 0.4 ^a
20	5.6 \pm 0.8 ^b	13.2 \pm 0.6 ^a	50 \pm 5 ^b	9.6 \pm 0.7 ^a
25	7.6 \pm 0.6 ^b	14 \pm 0 ^a	35 \pm 5 ^d	10.5 \pm 0.6 ^a
30	9.6 \pm 2.2 ^a	13 \pm 2 ^a	12 \pm 6 ^a	10.9 \pm 0.8 ^a
35	-	-	-	-
F- value	7.17**	1.67 ^{ns}	56.47***	4.18*
<i>S. ocymastrum</i>				
5	-	-	-	-
10	5.4 \pm 0.8 ^a	8.0 \pm 1.6 ^{ab}	13 \pm 2 ^{ab}	6.2 \pm 2.0 ^a
15	3.8 \pm 0.4 ^{ab}	10.4 \pm 0.4 ^a	40 \pm 4 ^c	6.0 \pm 1.8 ^a
20	2.0 \pm 0 ^b	8.6 \pm 0.8 ^{ab}	62 \pm 8 ^d	5.0 \pm 1.8 ^a
25	2.0 \pm 0 ^b	9.6 \pm 1.2 ^{ab}	55 \pm 4 ^d	5.4 \pm 1.2 ^a
30	2.6 \pm 1.1 ^b	5.6 \pm 0.4 ^{bc}	23 \pm 6 ^b	3.6 \pm 1.6 ^{ab}
35	3.2 \pm 0.5 ^{ab}	4.4 \pm 0.8 ^c	8 \pm 2 ^a	2.4 \pm 0.6 ^b
F- value	6.41**	16.42***	47.94***	10.45***

Different lowercase letters (column) show significant differences between the averages, F probabilities are indicated by symbols: ns, non-significant.

* P<0.05.

** P<0.01.

*** P<0.001.

Salinity effects

In seeds of that were treated by distillate water (0 mM NaCl) germination was the highest (*M. vulgare*-88%; *S. Incana*-68% and *S. ocymastrum*-64%). As is showed in table 2, germination capacity is significantly ($p<0.001$) affected by inclusion salt in the medium. Thereby, no seed germination in 132 Mm NaCl for *M. vulgare* and *S. incana*, however, seeds of *S. ocymastrum* can perpetuate at this concentration (18 %

germination) as well as decrease on Mean Time of Germination (MTG) values was recorded in high salinity levels which let suggested that this plant is more tolerant to salt stress. In this context, it has been widely reported that fresh water improved seed germination and NaCl induced compelled disruption on seed germination for both halophytes and glycophytes [18].

Table 2.

Germination characteristic variables of *M. vulgare*, *S. incana* and *S. ocymastrum* seeds in response to various NaCl concentrations (Mean \pm SE). IGD: Initial Germination Day / FGP: Final Germination Day / FGP: Final Germination Percentage / MTG: Mean Time of Germination.

NaCl concentration (mM)	Parameters			
	IGD (days)	FGD (days)	FGP (%)	MTG (days)
<i>M. vulgare</i>				
0	3.2 \pm 0.4 ^a	11 \pm 1.4 ^a	88 \pm 6 ^a	6.4 \pm 0.6 ^a
34	3.8 \pm 0.4 ^a	15 \pm 1.4 ^b	73 \pm 2 ^a	8.4 \pm 0.6 ^{ab}
68	4.6 \pm 1.0 ^a	17 \pm 0.8 ^b	55 \pm 4 ^b	9.9 \pm 0.65 ^b
102	9 \pm 1.6 ^b	17.4 \pm 1.2 ^b	22 \pm 2 ^c	13 \pm 1.2 ^c
136	-	-	-	-
F- value	8.22**	12.10**	98.97***	13.35**
<i>S. incana</i>				
0	6.4 \pm 0.6 ^a	14.7 \pm 0.6 ^a	68 \pm 7 ^a	9.4 \pm 0.6 ^a
34	7 \pm 1.8 ^{ab}	13 \pm 1.2 ^a	45 \pm 5 ^b	10 \pm 1.8 ^a
68	8 \pm 1 ^{ab}	13.7 \pm 0.8 ^a	30 \pm 5 ^c	10 \pm 1.8 ^a
102	9 \pm 1 ^b	13.4 \pm 1 ^a	14 \pm 3 ^d	9 \pm 1 ^a
136	-	-	-	-
F- value	3.06 ^{ns}	1.56 ^{ns}	56.10***	0.54 ^{ns}
<i>S. ocymastrum</i>				
0	2 \pm 0 ^a	8.6 \pm 0.5 ^a	64 \pm 6 ^a	6.0 \pm 1.0 ^a
34	2.6 \pm 0.8 ^a	7.4 \pm 0.4 ^b	38 \pm 6 ^b	4.6 \pm 1.2 ^{ab}
68	2.6 \pm 0.6 ^a	9.4 \pm 1.6 ^{ab}	30 \pm 4 ^{bc}	5.4 \pm 0.6 ^a
102	4 \pm 0.8 ^a	9.6 \pm 1.2 ^{ab}	22 \pm 5 ^c	4.4 \pm 1.2 ^b
136	4 \pm 1.2 ^a	10 \pm 0 ^c	18 \pm 6 ^c	4.8 \pm 1.6 ^b
F- value	1.8 ^{ns}	7.14**	23.26***	3.04 ^{ns}

Different lowercase letters (column) show significant differences between the averages, F probabilities are indicated by symbols:

ns, non-significant.

*p<0.05.

** P<0.01.

*** P<0.001.

Salt stress was remarkably affected *M. vulgare*'s FGD, IGP and MTG, and *S. ocymastrum*'s FGD. Seeds subjected to high salinity (0 vs. 102 NaCl Mm) onset germination more lately whatever species (3.2 vs. 9 d-*M. vulgare*; 6.4 vs. 9 d-*S. incana* and 2 vs. 4 d-*S. ocymastrum*) as well for Final Germination Day (FGD) which showed delay to achieve germination for *M. vulgare* (11 vs. 17.4 d) and *S. ocymastrum* (8.6 vs. 10 d) seeds. Salinity compromise germination patterns by I.) reducing the absorption of water or II) the accumulation of Na⁺ and Cl⁻. These ions affect the balance of nutrient absorption and cause a toxic effect [19]. Salinity usually prevent germination by induced dormancy mechanism; however at such unfavorable condition seeds should maintain viability in a given time up to establishment of conducive plant growth conditions, thereby leave at least one offspring in order to perpetuate [20]. Lastly, failure germination on moderate saline treatments is absolutely presumed as a common trait of glycophyte that caricaturizes our studied species. This paper supplies important information

to assist conservation biologist to undertake appropriate decisions for plant conservation and soil management.

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